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Growth Performance and Nutritional Value of *Chlorella ellipsoidea* and *Moina* sp. Cultured in a Bioreactor Tank System

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ABSTRACT

In this study, the impact of light-emitting diodes (LED) on cultivation of the microalga *Chlorella ellipsoidea* within a bioreactor system was analyzed. The study applied two different illumination systems, a tank illuminated with natural light (CT1 group) and a tank illuminated using LEDs (CT2 group). Statistical analysis revealed no significant difference (P>0.05) in the cell counts between CT1 and CT2 groups over the cultivation period, with maximum cell densities of 7.0 x 10^7 and 8.8 x 10^7 cells mL^{-1}, respectively. The cell size and amino acid content of *C. ellipsoidea* did not differ significantly between the groups. Fatty acids predominantly comprised linoleic acid and oleic acid, with significantly (P<0.05) higher quantities observed in the CT2 group. To evaluate the transfer of nutrients to zooplankton, *C. ellipsoidea* from the two groups were fed to *Moina* sp. (CT1 → MT1, CT2 → MT2). After two days, both *Moina* sp. groups had similar protein levels (64%), but they differed in fat content: 3.41% in MT1 and 6.90% in MT2. In summary, the cultivation of *C. ellipsoidea* under blue LED light resulted in higher levels of fat and fatty acids compared to cultivation under natural light. Additionally, this system was effective in transmitting the nutritional value to zooplankton. In conclusion, the results indicate that bioreactor tanks with blue LED have the potential to produce *C. ellipsoidea* and *Moina* sp. suitable for small-scale aquaculture and ornamental fish farmers. However, the choice of the LED cultivation system should be made judiciously, considering its optimal utilization during periods of low natural light to achieve the best cultivation results.

INTRODUCTION

Chlorella sp. is an alga that holds significant importance due to its diverse applications in various fields: it is utilized as a nutritional supplement for human consumption, as a source for biofuel production **(Khoeyi *et al*., 2012; Severes *et al*., 2017)**, and it plays a crucial role as a feed source for zooplankton, aquatic animals, and larvae in aquaculture **(Dhont *et al*., 2013; Lan *et al*., 2022; Joshua *et al*., 2024)**. Owing

to its remarkably good nutritional value, it is commonly used as a feed for *Moina* sp., a type of zooplankton that is an essential live feed for raising many species of aquatic animals **(Thewaratmaneekun *et al*., 2006)**. In Thailand, *Chlorella* sp. cultivation is carried out in both open systems, such as cement ponds (approx. 5x10 meters) for large-scale production **(Thewaratmaneekun *et al*., 2006)**, and closed systems within laboratories. Open-pond microalgal culture systems face multiple challenges, including susceptibility to contamination by invasive microorganisms, low microalgal biomass yield, and difficulties in controlling environmental conditions **(Wang *et al*., 2013; Narala *et al*., 2016)**. Therefore, closed-system cultivation plays a greater role in algae cultivation, as it allows for higher biomass production per unit area, better control of algae cleanliness by reducing environmental contamination, utilizes smaller production areas, and incorporates natural factor control systems conducive to algal growth **(Razzak *et al*., 2024)**. For instance, the use of artificial lighting systems allows for more precise control over the duration of light exposure, significantly enhancing suitability compared to open pond cultivation methods.

Green microalgae, including *Chlorella* sp., contain chlorophyll-a and chlorophyll-b in a 3:1 ratio, with absorption peaks in the blue and red regions of the spectrum. The chlorophyll-a absorption peaks around 430nm (blue) and 660nm (red), and the chlorophyll-b absorption peaks around 460nm (blue) and 630nm **(Devaraja *et al*., 2017)**. Previous studies found that the use of light-emitting diode (LED) lighting in closed algal growth systems is an efficient method for converting electric energy into light energy for microalgal production, including *Chlorella* sp. **(Pattanaik *et al*., 2018)**. LEDs emit monochromatic light with highly saturated colors, which are beneficial for microalgal growth, so they offer a promising light energy source for improving the economic feasibility of microalgal-based products grown in closed systems. LED lights can provide a spectrum across multiple ranges depending on the type and color of LED bulbs, including the red and blue spectra that algae require for photosynthesis. Therefore, LED lights have been applied in algae cultivation **(Kim & Rhee, 2017)**, and many studies focused on the use of LEDs as an alternative light source to natural sunlight in the cultivation of *Chlorella* sp. Notably, in comparison to white, green, or red LED lights, blue LED light in an indoor setting has shown significant positive effects on the biomass production of *C. ellipsoidea*, comprising the cell density, cell dry weight, protein content, lipid content, and cell size **(Baidya *et al*., 2021)**. Similarly, continuous exposure to blue LED light for a 24:0 hour light-dark cycle has shown positive effects on the cell number of *Chlorella* sp. **(Kamolrat *et al*., 2023)**. Therefore, the use of blue LED lights for *Chlorella* sp. cultivation presents an intriguing alternative to natural sunlight and fluorescent tubes. To assess the efficacy of blue LED lights in the cultivation of *Chlorella* sp., a study was conducted to investigate their impact on growth rates and nutritional quality of the algae.

The focus of previous algae cultivation studies in bioreactor systems has largely been on producing high-quality algae or bioenergy **(Hu *et al*., 2008; Yan & Zheng *et al*., 2014; Severes *et al*., 2017; Pattanaik *et al*., 2018)**. However, this study aimed to apply bioreactor systems for cultivating algae intended for aquaculture purposes. Furthermore, the development of a small-scale bioreactor system aims to enhance accessibility for small-scale farmers. A part of the utility of *Chlorella* sp. lies in its application in aquaculture; serving not only as direct feed for aquatic animals but also as a source of nutrition for zooplankton, particularly *Moina* sp. (order Cladocera: family Moinidae). Due to its small size, slow swimming speed, ease of culture, short life cycle, high nutritional content, and abundance of digestive enzymes **(Taghavi *et al*., 2013)**, this zooplankton is a suitable live food for larval stages of fishes in freshwater aquaculture and ornamental fish breeding **(Kumar *et al*., 2005; Taghavi *et al*., 2013)**. *Moina* sp. exhibits filter-feeding behavior, utilizing a mechanism wherein suspended particles are captured as water flows through its mouth. This feeding mechanism involves specialized structures known as setae, arranged in rows along the sides of paired appendages, acting as filters. Consequently, *Moina* sp. mostly receives nutrients from ingested food particles, indicating that the quality of its nutrition depends on the quality of available food sources. If *Moina* sp. inhabits water bodies with inadequate nutrition, it may suffer from nutrient deficiencies. *Chlorella* sp. cultivated in a quality-controlled feeding system provides all essential nutrients necessary for the growth of *Moina* sp., comprising carbohydrates, proteins, and lipids **(Aly *et al*., 2023)**. Hence, the cultivation of *C. ellipsoidea* in closed systems or bioreactors emerges as a viable option to enhance the quality and cleanliness of algae used to feed *Moina* sp. To validate the suitability of algae cultivated in bioreactors with blue LED lights as food for zooplankton, this study investigated the growth rate of *C. ellipsoidea* in bioreactors compared to those grown under natural light conditions. Additionally, we assessed the cell size and examined the amino acid and fatty acid profiles of both groups. Moreover, water quality was evaluated during the algae cultivation to determine the suitable release time for introducing *Moina* sp. into the bioreactor tanks. Furthermore, we conducted experiments designed to assess the suitability of bioreactor-grown algae as a fee source for *Moina* species. *Moina* sp. were fed *C. ellipsoidea* from both groups and their nutritional quality was evaluated. This comprehensive investigation aimed to elucidate the nutritional transfer from algae to zooplankton, providing insights into the potential benefits of utilizing bioreactor cultivated algae as a sustainable feed source in aquaculture.

MATERIALS AND METHODS

1. The experimental tank setup

A 20-liter container was used to set up a prefabricated culture tank. The tank was equipped with 30 blue LED lights (Module LED model 5630; 1.5 W, 150 lumens; China)

for illumination, and a water vortex system with a water pump. The lights were managed by an automatic controller. The light energy was measured as units of photosynthetic photon flux density (PPFD) and it was adjusted to 355-370µmol m^{-2} s^{-1}.

2. Preparation of algal samples

An algal sample of *C. ellipsoidea* TISTR 8260 was obtained from a cultivar on a Plankton Laboratory, Department of Fisheries, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, Thailand. The initial cell number of the algae used in the experiment was 2.1×10^5 cells mL^{-1}.

3. Preparation of the culture medium

The algal culture medium was composed of 0.5g L^{-1} Urea (CH_4N_2O), 0.2 g L^{-1} phosphorus pentoxide (P_2O_5), and 0.2 g L^{-1} potassium oxide (K_2O). Glucose ($C_6H_{12}O_6$) was added as a source of carbon at 0.5g L^{-1} and lime (CaO) was added at 0.4g L^{-1}. The medium was mixed using distilled water as a solvent to obtain a total volume of 15L.

4. Experimental method

In the experiment, the algae cultivated in an outdoor environment, exposed to natural sunlight with a 12:12h light:dark cycle comprised the CT1 group (Fig. 1A), whereas the algae cultivated indoors under an LED light with a 24:0h light:dark cycle **(Kamolrat *et al*., 2023)** comprised the CT2 group (Fig. 1B). The experiment was carried out using a completely randomized design with three replications.

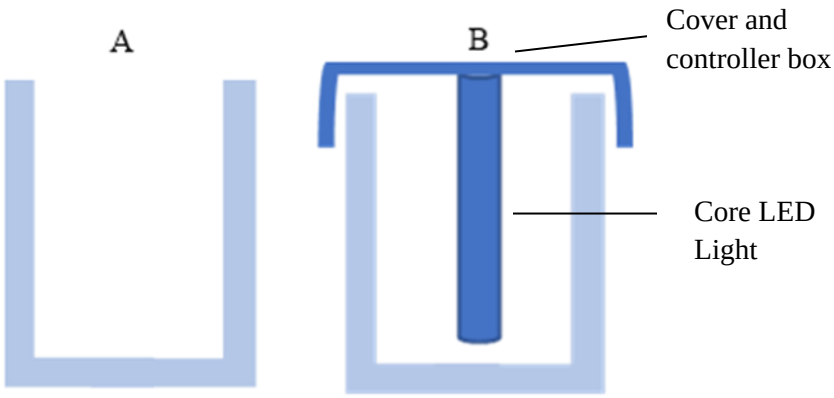

Fig. 1. Culturing tanks: (A) CT1 is natural light, and (B) CT2 is LED light

5. *Chlorella ellipsoidea* data collection

Algal samples, collected at a volume of 5mL, were obtained daily at 11:00 am during the entire culturing period. Subsequently, algal cell counts were conducted using a hemocytometer (1/10 mm; BOECO; Germany), as described by **Absher (1973)**. The cell

size analysis involved collecting algae samples during the exponential growth phase. Droplets of the algae sample, each measuring 1ml in volume, were prepared and examined under a microscope with a magnification of 40x. The images were captured using camera (EOS 800D) and EOS Utility software, and the algae size was measured in micrometers. The total algal cells mL^{-1} were determined using the following formula:

$$Total\ cells/ml\ = \frac{grid\ (1 \pm 2 \pm 3 \pm 4 \pm 5)}{5}\ x\ 0.25\ x\ 10^6$$

6. Water quality data collection

The evaluation of *C. ellipsoidea* culture quality encompassed continuous monitoring of water quality parameters throughout the algae cultivation period, spanning from the lag stages to the death phase. This comprehensive assessment aimed to identify the optimal timeframe for both peak algae proliferation and the suitable period for *Moina* species inoculation into the experimental tanks. The chemical parameters of water quality, were obtained daily at 11:00 am during the entire culturing period including pH, total ammonia nitrogen (TAN), and alkalinity (Alk), were daily examined using water analysis kits (Icon, Thailand).

7. Preparation of *Moina* sp. samples

Moina sp. samples were obtained from a breeding stock for commercial purposes at the Aquaculture Research Station, Nong Han Chaloem Phrakiat Research Institute in Thailand. In the initial phase of the study, *C. ellipsoidea* was cultured until the algae entered the exponential phase, typically occurring on days 6-7 of the cultivation process. Water parameters in the algae cultivation tanks were monitored, and pH, Alk, and TAN values were measured using water analysis kits (Icon, Thailand). The accepted water quality criteria were set within the pH range of 7-8, Alk not exceeding 120ppm, and TAN levels not surpassing 2ppm **(Benider *et al*., 2002; Xi *et al*., 2005; Thewaratmaneekun *et al*., 2006)**. Subsequently, *Moina* sp. were introduced into the experimental tanks, with 50 grams (wet weight) added to each CT1 and CT2 tank, along with aeration. *Moina* sp. were allowed to consume algae for two hours. For sample collection, after feeding, aeration was turned off to allow *Moina* sp. to gather at the water surface. A fine mesh net was used to collect samples from multiple points in the tank. Water was gradually drained through the outlet, with a fine mesh placed over the outlet to prevent the loss of *Moina* sp. during drainage. The collected *Moina* sp. were transferred to a pre-weighed container, and their wet weight was measured. A total of 20-30 grams (wet weight) was collected from each tank. After feeding, 20 grams (wet weight) of live *Moina* sp. were placed in sealed plastic bags and stored in a freezer at -20°C for subsequent nutritional analysis.

8. Nutritional analysis

The preparation of *C. ellipsoidea* samples for nutritional analysis involved inducing flocculation in the algae in the cultivation tanks using alum. The flocculated algae were collected, placed in containers, and stored in a refrigerator at 4°C. Subsequently, the algae were freeze-dried using a Freeze Dryer, resulting in a powdered, dried algae form. The nutritional content of the algae was assessed by measuring the amino acid content using High-Performance Liquid Chromatography (HPLC) following the method of **Bartolomeo and Maisano (2006)**. Additionally, the analysis of lipid content was performed using Gas Chromatography (GC; Agilent 8890 GC System; USA) according to the protocol outlined by **Bruchmann *et al*. (2012)**.

For the *Moina* sp., samples stored in a -20°C freezer were used for nutritional analysis. The assessment included measuring moisture, crude protein, fat, ash, and crude fiber content. Crude protein content was determined by Kjeldahl technique. Moisture, ash, and crude fiber content were analyzed using the **AOAC (2000)** method, while the fat content was determined using the Gerhardt Soxtherm methods **(Zygler *et al*., 2012)**.

9. Statistical analysis

The data on the growth rate, cell size, water quality, amino acid and fatty acid contents, and nutritional value of *Moina* sp. were analyzed and comparisons were made using a t-test at the 95% confidence level in SPSS version 20.

RESULTS

1. The growth rate of *C. ellipsoidea*

The initial cell density of *C. ellipsoidea* was 2.1×10^5 cells mL^{-1}. During the log growth phase, 2 to 4 days following the algae inoculation into the experimental tanks, the two groups exhibited significantly different growth patterns ($P < 0.05$; all groups repeated), with CT2 exhibiting a higher growth rate. At the end of the stationary phase (days 5 to 7) the maximum cell counts were 7.0×10^7 cells mL^{-1}in CT1 and 8.88×10^7 in CT2, but they did not differ statistically (Fig. 2). In the death phase, occurring after day 8 of cultivation, significant differences were observed between the two test groups. CT1 exhibited a continuous decrease in cell count, whereas CT2 experienced a slight increase in cell count on day 10, followed by a decline. Both test groups were cultured for a total of 12 days.

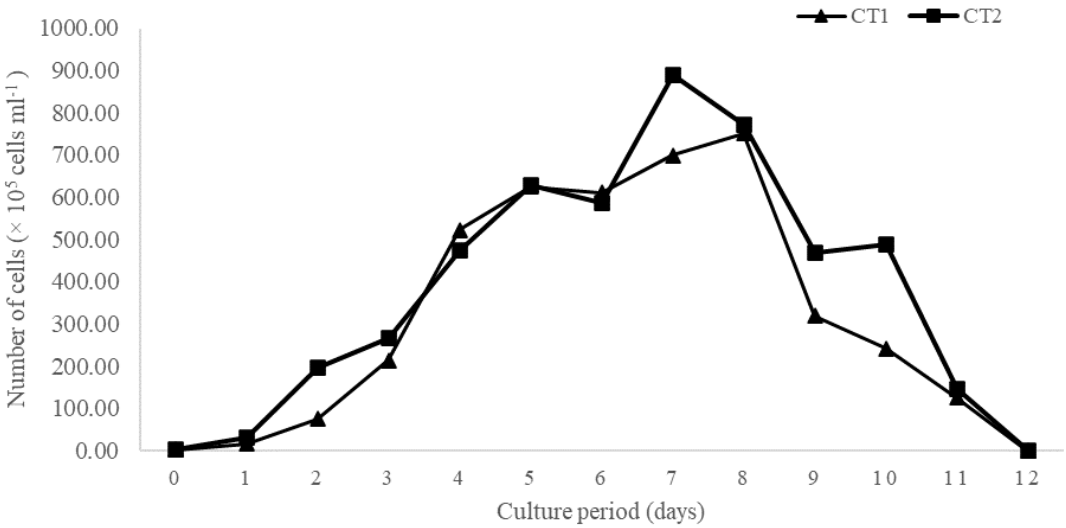

Fig. 2. Growth results of *C. ellipsoidea* in groups CT1 and CT2 during the 12 days

2. The cell size of *C. ellipsoidea*

Cell size measurements of *C. ellipsoidea* were conducted using a microscope with a magnification of 400×. Thirty cells were randomly selected and measured per experimental group, with three replicates for each group. Samples were collected during the stationary phase of algae cultivation. The average cell size of *C. ellipsoidea* in the CT1 group was 6.14 ± 0.7µm, and in group CT2 it was 5.92 ± 0.76µm (Fig. 3). The cells in both groups had similar color and shape. Statistical analysis revealed no significant difference between the two groups.

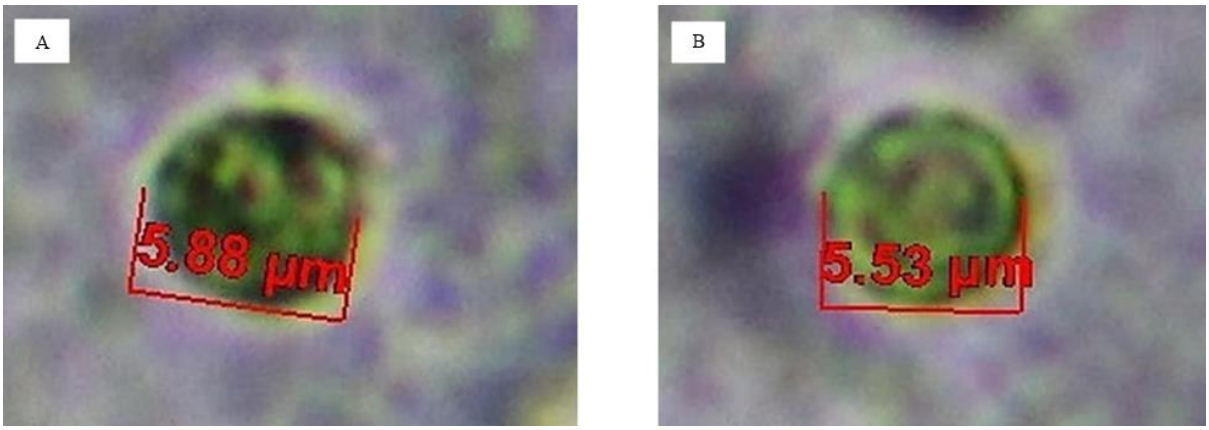

Fig. 3. Cell sizes of *C. ellipsoidea* in CT1 (A) and CT2 (B) groups

3. Water quality

The evaluation of water quality was employed to assess the optimal timing for the release of *Moina* sp. into the algae cultivation tank, concurrent with the algae growth phases. We focused on three water quality parameters for which the optimal values for *Moina* sp. sustenance are known: pH range of 7-8, alkalinity <200, and TAN <2. Throughout the cultivation period, both experimental groups exhibited pH values ranging from 7.3 to 8.5, with no statistically significant differences (Fig. 4). Alkalinity levels increased initially, particularly during the log phase of algae cultivation, reaching maximum values of 385.33ppm for CT1 and 374ppm for CT2, gradually decreasing during consecutive days until the stationary phase of the experiment, when they were 79.3 and 85.0ppm, respectively, with no significant differences (Fig. 5). The TAN levels increased to 3.0 and 2.33ppm, respectively, during the log phase, but they remained under 0.5ppm during the stationary phase in both groups. In the death phase, TAN levels increased to 3.0ppm for both groups, with no statistically significant differences (Fig. 6). Therefore, the appropriate phase for *Moina* sp. release into the algae cultivation tank was determined to be the stationary phase (day 7), when algae cell number and water quality were suitable for the survival of *Moina* sp.

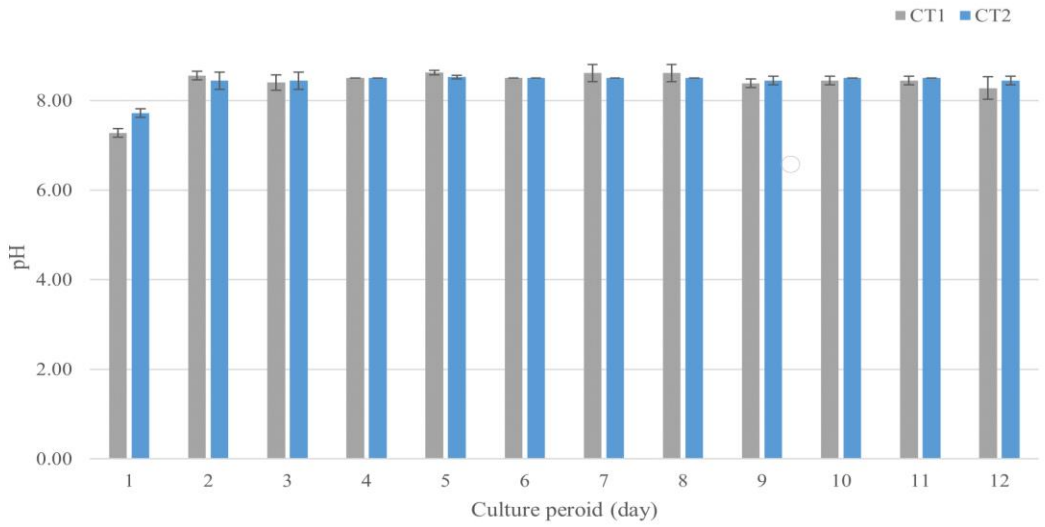

Fig. 4. pH values during 12 days of *C. ellipsoidea* cultivation: CT1 and CT2

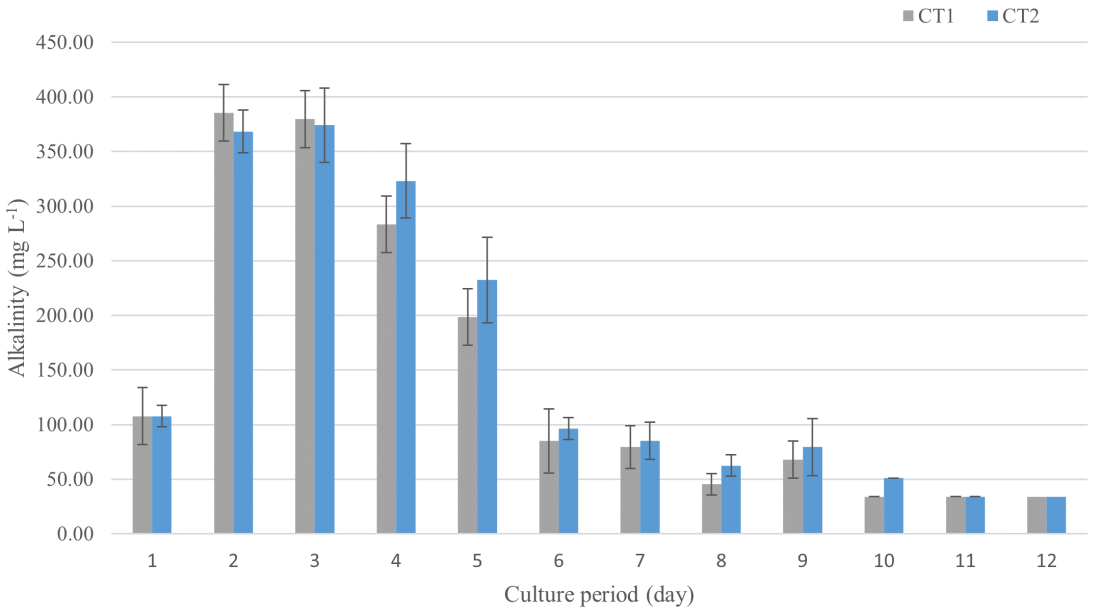

Fig. 5. Alkalinity levels during 12 days of *C. ellipsoidea* cultivation: CT1 and CT2

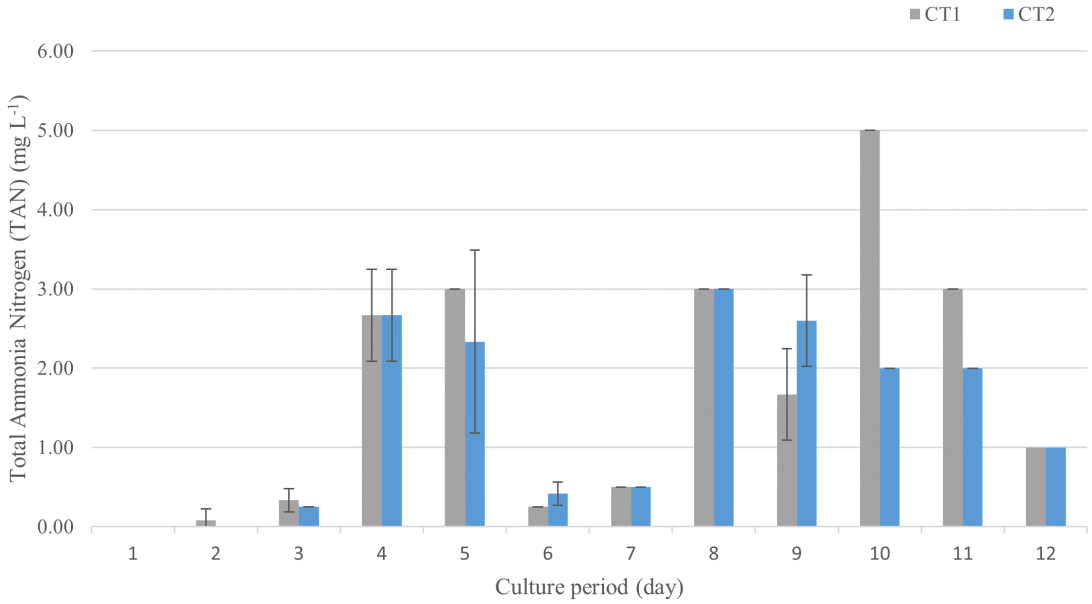

Fig. 6. TAN levels during 12 days of *C. ellipsoidea* cultivation: CT1 and CT2

4. Amino acids

The nutritional analysis of *C. ellipsoidea* was conducted to determine the amino acid content using the HPLC analysis. The amino acid levels did not significantly differ between the CT1 and CT2 groups ($P > 0.05$), as shown in Table (1).

Table 1. Amino acids in *C. ellipsodea* (g/100g) grown in different systems

Amino acid	Treatments	
	CT1	CT2
Aspartic acid	1.06±0.05	1.07±0.09
Threonine	0.54±0.01	0.54±0.04
Serine	0.47±0.01	0.47±0.04
Glutamic acid	1.29±0.07	1.21±0.12
Glycine	0.64±0.02	0.63±0.06
Alanine	0.80±0.01	0.78±0.07
Valine	0.68±0.04	0.68±0.06
Cystine	0.12±0.01	0.10±0.02
Methionine	0.23±0.01	0.24±0.02
Cystine+Methionine	0.35±0.03	0.33±0.04
Isoleucine	0.47±0.03	0.45±0.04
Leucine	0.89±0.03	0.92±0.08
Tyrosine	0.38±0.04	0.36±0.04
Phenylalanine	0.58±0.01	0.61±0.06
Lysine	0.76±0.01	0.72±0.08
Histidine	0.20±0.00	0.19±0.01
Arginine	0.63±0.05	0.62±0.05
Proline	0.48±0.07	0.49±0.05
Total amino acid	10.19±0.47	10.02±0.94

5. Fatty acids

The analysis of fatty acids in *C. ellipsoidea* using gas chromatography revealed that the algae primarily contained C16:0 and C18 fatty acids. The CT1 group had significantly higher levels of C16:0 and C18:3n3 fatty acids compared to the CT2 group ($P< 0.05$). Conversely, the CT2 group exhibited significantly higher levels of C16:1, C18:0, C18:1n9, and C18:2n6 fatty acids than the CT1 group ($P< 0.05$). When categorized, the CT1 group had higher n-3 fatty acids, whereas the CT2 group had higher n-6 fatty acids. The results showed a significant difference in total fatty acid content between the two experimental groups, with the CT2 group exhibiting a higher total fatty acid content than CT1 group (Table 2).

Table 2. Fatty acids in *C. ellipsoidea* from the two different growth systems

Major fatty acid (% of the total oil content)	Treatments	
	CT1	CT2
Ginkgolic acid (C15:1)	1.96±1.5	3.15±0.66
Palmitic acid (C16:0)	21.63±3.5*	18.96±1.57
Palmitoleic acid (C16:1)		1.99±0.01*
Stearic Acid (C18:0)		1.81±0.21*
Oleic acid (C18:1n9)	6.55±1.36	8.99±3.08*
Linoleic acid (C18:2n6)	12.70±3.37	24.65±3.45*
Alpha linolenic acid (C18:3n3)	33.41±1.36*	20.55±1.45
SUM of fatty acid	76.25±0.21	80.1±2.72
Total n-3	33.41±1.36*	20.55±1.45
Total n-6	12.70±3.37	24.65±4.45*
n3/n-6	2.63±1.29*	0.83±1.27
Total fat (%)	0.10±0.04	0.13±0.01
n-3 in sample (%)	0.06±0.01	0.08±0.01
n-6 in sample (%)	0.01±0.00	0.03±0.01
Moisture (%)	78.68±0.01	78.11±0.01

* Values within the same row with different superscripts are significantly different ($P < 0.05$).

6. Analysis of the nutritional value of *Moina* sp. fed with *C. ellipsoidea*

Analysis of fresh *Moina* sp. samples weighing 20 grams revealed no significant differences in moisture contents: 94.41±0.69% in MT1 and 95.56±1.55% in MT2. Analyzing the dried weight of *Moina* sp. revealed that the protein content also did not differ significantly between the two groups: 64.19±0.20% in MT1 and 64.89±5.20% in MT2. However, the content of fat, ash, and fiber differed significantly between the two groups. MT2 had a higher fat content compared to MT1: 6.90±0.70% vs. 3.41±0.13%, respectively. However, the ash content was higher in MT1 than in MT2: 12.70±0.98% vs. 7.96±0.61%, respectively, as was the fiber content: 3.69±0.38% vs. 2.47±0.65%, respectively (Table 3).

Table 3. Proximate analysis of the nutritional value of *Moina* sp. fed *C. ellipsoidea* from the two different growth systems

Proximate composition (%)	Treatments	
	MT1	MT2
Moisture	95.56±1.55	94.41±0.69
Dry matter	4.44±1.55	5.59±0.69
Ash	12.7±0.98*	7.96±0.61
Protein	64.89±5.20	64.19±0.20
Fat	3.41±0.13	6.90±0.70*
Fiber	3.69±0.38*	2.47±0.65

* Values within the same row with different superscripts are significantly different ($P < 0.05$).

* The moisture content is determined based on the wet weight, whereas the analysis of ash, protein, fat, and fiber involves assessing values derived from the dry matter.

DISCUSSION

The CT2 group exhibited a higher cell count during the lag phase, but there were no significant differences between the two groups during the exponential growth phase. During the early growth stages, algae adapt to their environment and rapidly grow when suitable conditions are met. The CT2 group exhibited a better growth performance during this phase due to the continuous growth stimulation under the 24-hour LED light regime. In the death phase, CT2 exhibited a slower rate of decline. We hypothesize that this may be due to a higher amount of algal cells in the circulation, which provided nutrients after death, coupled with the continuous 24-hour light stimulation. Together, these two factors provided a sufficient stimulus for the short-term growth during days 9 and 10 of the experiment (Fig. 2). The blue light spectrum, ranging from 400 to 500nm, is crucial for the photosynthetic stimulation of chlorophyll-*a* and chlorophyll-*b* in *C. ellipsoidea* cells. Therefore, providing suitable and continuous blue light spectra along with sufficient nutrients enhanced the growth of *C. ellipsoidea*, resulting in a higher growth rate in the CT2 group compared to the CT1 group. There were no significant differences in water alkalinity between the two groups. However, there was a correlation between the TAN levels and cell count during the late stage of cultivation. After day 8 of the experiment, both experimental groups of *C. ellipsoidea* entered the death phase. The cell count consistently decreased in group CT1, corresponding to the increasing level of TAN during the same period. Conversely, in group CT2, there was a slight increase in cell count around day 10, accompanied by a decrease in TAN levels (Fig. 6). We attribute this to the fact that CT2 had a higher cell count when it reached the death phase, which could contribute to the conversion of cells into TAN. On day 8 of the experiment, both groups had the same TAN level (3ppm). However, due to the continuous light exposure received by CT2, coupled with the increased TAN level used as a nutrient source for algae, the algae were able to temporarily continue growing. This is beneficial for the cultivation of aquatic animals, allowing for a longer culture and harvest period for the algae. When

considering the optimal time frame for introducing *Moina* sp. into the algae cultivation tank, on the 7th day of the experiment, the cell count of algae in both experimental tanks reached its peak and stabilized, with TAN levels remaining at 0.5ppm. The pH levels of both experimental groups remained within the range of 7.3-8.5 throughout the cultivation period. Both TAN and pH values influenced the NH_3/NH_4^+ levels, maintaining the conditions non-toxic to *Moina* species. Consequently, day 7 was identified as the suitable release date for *Moina* sp. into the algae cultivation tank, ensuring sufficient food availability and optimal water quality conditions for their survival in the experimental tanks.

In measuring cell size and amino acid quantities in *C. ellipsoidea* from both groups, there was no statistically significant difference. The synthesis of amino acids in algae depends on the nutrients in the water and the activity of chlorophyll-*a* and -*b*, which extract nitrogen from NH_4^+ and CO_2 to form cells **(Yan & Zheng, 2014)**. Despite the different light conditions, CT1 and CT2 groups did not differ in the content of amino acid levels. This indicates that the functioning of chlorophyll-*a* and -*b* under LED blue light conditions was efficient in synthesizing amino acids and cell formation comparable to natural light conditions. Regarding the fatty acids, the accumulation of palmitic, oleic, and linoleic acids was higher compared to other types of fatty acids. Algae generally synthesize fatty acids as building blocks for the formation of various lipids. The majority of synthesized fatty acids in Chlorophyceae are C16:0 and C18:1. Additionally, when *Chlorella* sp. algae are grown under unfavorable conditions, such as photo-oxidative stress or nutrient starvation, they accumulate increased amounts of fatty acids, especially C16 and C18 compounds, containing palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linolenic acids (C18:2), or biodiesel-related fatty acids **(Hu *et al*., 2008; Knothe, 2008)**. In this experiment, several factors created unfavorable conditions for the normal growth of algae. For example, the cultivation tanks had an opaque cylindrical shape and light conditions differed between the CT1 and CT2 groups. The CT1 group absorbed natural light from the top of the tank, which resulted in the algae at the bottom of the cultivation tanks not receiving light evenly. In contrast, the CT2 group absorbed blue LED light from the center of the cultivation tank and had a water circulation system, allowing the algae to receive light more uniformly. Another cofounding factor might be oxygenation in the CT2 group, where the tank cover was closed, so oxygenation was achieved through small apertures which allowed air circulation in the tank. Additionally, the heat generated by the light bulbs was dissipated into the water within the tank, possibly creating a stressful environment for *C. ellipsoidea*. Furthermore, each type of LED bulb has different light wavelengths, intensity, and quantity, which affects the light absorption of the algae at different levels. This has implications for the production of biochemical compounds, including different fatty acid profiles **(Wang *et al*., 2007)**. A study by **Kim *et al*. (2016)** on the impact of light wavelengths from LED bulbs on the cultivation of *C. vulgaris* found that the predominant fatty acids in

C. vulgaris cultivated under LED light were oleic acid and linoleic acid, which significantly influenced the properties of biodiesel. Further research should be conducted to investigate factors that affect stress levels and their impact on fatty acid production in algae. Nutritional analysis of *Moina* sp. from both experimental groups produced results consistent with the analysis of two *C. ellipsoidea* groups. Specifically, MT1 and MT2 did not differ significantly in protein content, but MT2 had higher fat levels than MT1. These fats primarily consisted of fatty acids, constituting approximately 80% of the total fat content. Therefore, the nutritional value derived from the analysis of *Moina micrura* stems from the nutritional value obtained from the entire biomass of *C. ellipsoidea*. Aside from absorbing minerals through their diet, planktonic animals can absorb some of the minerals present in the water through their gills and intestinal walls **(Habib *et al*., 1997)**. A study on the use of palm oil mill effluent (POMED) for cultivating *C. vulgaris* found that *C. vulgaris* exhibited significantly higher levels of essential minerals when grown in water with increased POMED concentrations. Similarly, *M. micrura* accumulated significantly higher levels of essential minerals when fed with algae grown in POMED **(Habib *et al*., 2003)**. It is evident that the nutritional value derived from the food consumed by *Moina* sp. directly influences the nutritional content accumulated within *Moina* sp. themselves.

Therefore, it can be concluded that groups cultivated under LED light with a specific wavelength range exhibited differences in the synthesis of fats and fatty acids compared to those cultured under natural light. This underscores the observation that factors affecting the production and accumulation of fats are similar to those influencing the accumulation of essential oils in plants: environmental conditions, light period, light intensity, and light spectrum **(Li & Kubota, 2009; Fernandes *et al*., 2013; Shafiee-Hajiabad *et al*., 2016; İzgı *et al*., 2017)**. The light spectrum plays a significant role in controlling plant morphology, growth processes, and photosynthetic activities **(Wang *et al*., 2016)**. Additionally, many plant species can adapt to different light conditions by altering their essential oil content, which might be one of the mechanisms through which plants respond to stress **(Zhang *et al*., 2003)**. Factors such as temperature, light intensity, and air pressure, have varying effects on different plant species. A limitation of the study is the absence of recorded environmental factors, such as temperature changes, which could potentially impact the production of both *C. ellipsoidea* and *Moina* species. These factors can also impact the mortality and cause nutritional deficiencies of *Moina* sp. during cultivation. It is also crucial to consider the appropriate amount of *Moina* species. supplementation. As a zooplankton filter feeder, *Moina* sp. has the ability to rapidly consume algae. Therefore, after introducing *Moina* sp. into the cultivation tank, continuous monitoring of cell count changes in *C. ellipsoidea* is necessary to prevent nutrient deficiencies and to ensure the timely harvest of *Moina* sp. The results of this study demonstrated that both *C. ellipsoidea* and *Moina* sp. could be successfully cultivated within a bioreactor, yielding results comparable to the natural light cultivation.

Future research should focus on controlling various factors during the *C. ellipsoidea* cultivation, such as studying the spectrum and wavelength of the available light and optimizing the nutritional formula to enhance the nutritional value provided to *Moina* species. Analysis of amino acids and fatty acids within the *Moina* sp. should be conducted to determine their precise quantities. Additionally, the development of an efficient cultivation tank is necessary to improve the overall effectiveness of the cultivation process. The continuation of this research should involve utilizing *Moina* sp. in the cultivation of ornamental fish or aquatic animal larvae.

CONCLUSION

The study successfully cultivated *C. ellipsoidea* in the designed bioreactor system. Under LED light, *C. ellipsoidea* showed increased cell production efficiency without significant differences in cell size between groups. Amino acid composition was similar between groups, but CT2 had significantly higher lipid levels, especially C16 and C18 fatty acids. *Moina* sp. mirrored the nutritional profile of *C. ellipsoidea*, with MT2 showing higher fat content than MT1. Water quality on day 7 was suitable for the survival and biomass accumulation of MT1 and MT2 in CT1 and CT2 tanks. This indicates the potential of blue LED light bioreactors for cultivating both algae and zooplankton.

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